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TEX101 is shed from the surface of sperm located in the caput epididymidis of the mouse

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Summary

It is generally believed that cell-to-cell cross-talk and signal transduction are mediated by cell surface molecules that play diverse and important regulatory roles in spermatogenesis and fertilization. Recently, we identified a novel plasma membrane-associated protein, TES101-reactive protein (TES101RP, or TEX101), on mouse testicular germ cells. In this study, we investigate *Tex101* mRNA expression in the adult mouse testis using *in situ* hybridization, and we examine the fate of TEX101 during sperm transport by immunohistochemical and Western blot analyses. *Tex101* mRNA was expressed in a stage-specific manner in spermatocytes and in step 1–9 spermatids of the testis, but not in spermatogonia. Although the TEX101 protein remained on the cell surfaces of step 10–16 spermatids and testicular sperm, it was shed from epididymal sperm located in the caput epididymidis. The results of this study provide additional information on germ cell-specific TEX101 expression during spermatogenesis and post-testicular sperm maturation.

Keywords: Epididymis, Immunohistochemistry, Mouse TEX101, Sperm maturation, Testis

Introduction

Spermatogenesis comprises a series of complex events through which germ cells differentiate in the testis. As a result, type A_{single} spermatogonia, which are considered to be adult stem cells, are transformed into spermatozoa. Sperm generation occurs continuously in the seminiferous tubules and involves cell-to-cell and cell-to-extracellular matrix cross-talk as well as cell signalling via cell surface molecules (reviewed in Fujisawa, 2001; Siu & Cheng, 2004). In addition, sperm maturation (the acquisition of fertilizing ability) takes place outside the testis, i.e., during sperm transport through the male and female genital tracts. Initial maturation occurs in the epididymis and is characterized by sperm-to-transport pathway cross-talk via cell surface molecules (reviewed in Dacheux *et al*., 2003). Therefore, the identification and characterization of stage-specific cell surface molecules on germ cells during testicular spermatogenesis and post-testicular maturation would further our understanding of the differentiation and fertilization of germ cells.

Recently, we identified a novel 38 kDa testicular protein (the TES101-reactive protein, TES101RP), which in adult mice is expressed predominantly on the cell surfaces of spermatocytes and spermatids, but not on the surfaces of Sertoli cells or interstitial cells, which include Leydig cells (Kurita *et al*., 2001). TES101RP is now designated as protein TEX101 in the Mouse Genome Informatics (MGI) database (MGI ID: 1930791) and is referred to as TEX101 in this report. Recently, TEX101 homologues have been discovered in two species. The rat homologue of mouse TEX101, TEC-21, has been detected in the rat basophilic leukaemia

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cell line RBL-2H3 (Halova *et al*., 2002; Entrez Gene ID: 207113), and the human homologue (Entrez Gene ID: 83639) has been identified as a novel cancer/testis antigen in small cell lung cancer (Tajima *et al*., 2003). Although the molecular cloning of the mouse *Tex101* gene and immunocytochemical characterization of the TEX101 protein in the adult mouse testis have been reported (Kurita *et al*., 2001), little information is available on the post-testicular modification of murine TEX101.

In the present study, the fate of TEX101 expressed on the surface of mouse sperm during sperm transport was investigated by immunohistochemical and Western blot analyses. In addition, the stage-specific expression of *Tex101* in the adult mouse testis was confirmed by *in situ* hybridization. The results provide additional information on the expression of the unique germ cell surface molecule TEX101 during spermatogenesis and post-testicular sperm maturation.

Materials and methods

Animals

All animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals of Jichi Medical School and Nippon Medical School. Adult male and female BALB/c mice (8–12 weeks of age) were purchased from Japan SLC (Hamamatsu, Japan). The mice were housed and bred at 25 °C with a 12 h/12 h light–dark cycle and were given food and water *ad libitum*.

In situ hybridization

In situ hybridization (ISH) was performed as described previously (Takayama *et al*., 2005). The testes from BALB/c mice were cut into small pieces and fixed for 2 h at room temperature in 4% paraformaldehyde in 100 mM sodium cacodylate buffer (pH 7.4) containing 5% sucrose. Tissue sections (8 μm in thickness) were cut with a Jung Frigocut 2800E cryostat (Leica, Nuccloch, Germany), mounted on round glass coverslips (13 mm diameter, no. 1 thickness; Matsunami, Osaka, Japan), coated with 2% 3-aminopropyltriethoxy-silane (Sigma Chemical, St Louis, MO), and then allowed to air-dry.

Two sense and two antisense probes were constructed, one pair for the N-terminal region of the *Tex101* cDNA sequence (antisense, 5′-CGGCCTGGATCGTCTTCCAGACTCAGGG-3′; sense, 5′-CCCTGAGTCTGGAAGACGATCCAGGCCG-3′) and one pair for the C-terminal region (antisense, 5′-CCGCCTCTCCTCCTTGAGAAACACAGCTCTTACTGGCC-3′; sense, 5′-GGCCAGTAAGAGCTGTGTTTCTCAAGGAGAGGCGG-3′). The oligonucleotide probes were biotinylated at their 5′ ends (TaKaRa Bio, Shiga, Japan).

Sections were treated with 1 μg/ml proteinase K (Merck, Darmstadt, Germany) for 10 min and then post-fixed in 0.1 M phosphate buffer (pH 7.6) containing 4% paraformaldehyde. The sections were pre-incubated in hybridization solution (see below) without oligonucleotide probe for 1 h at room temperature and then incubated in hybridization solution in a moist chamber at 37 °C overnight. The hybridization solution contained 50% deionized formamide, 10% dextran sulfate (Pharmacia Biotech, Uppsala, Sweden), 450 mM NaCl, 45 mM sodium citrate, 1× Denhardt's solution, 100 μg/ml salmon sperm DNA (Sigma), 125 μg/ml yeast RNA (Ambion, Austin, TX), and 1 μg/ml of each oligonucleotide probe. After hybridization, the solution was removed by washing with 2× SSC (1× SSC contains 150 mM NaCl, 15 mM sodium citrate) at 40 °C. Post-hybridization washes (40 °C) were carried out twice in 1× SSC for 10 min and twice in 0.5× SSC for 10 min. Next, the specimens were rinsed in phosphate-buffered saline (PBS). In order to visualize ISH signals, tyramide signal amplification followed by fluorescein-isothiocyanate-labelled streptavidin was employed as described previously (Takahashi *et al*., 1999). The sections were counterstained with Hoechst 33342 (Molecular Probes, Eugene, OR), mounted in ProLong anti-photobleaching medium (Molecular Probes), and examined with a Provis AX80TR microscope (Olympus, Tokyo, Japan). Controls consisted of addition of the antisense probe to sections that had been pretreated with 1 μg/ml RNase (Boehringer Mannheim, Mannheim, Germany), or addition of the sense probe to untreated sections.

RT-PCR

Total RNA was extracted from gonadal tissues using the Isogen reagent (Nippon Gene, Tokyo, Japan), according to the manufacturer's instructions. cDNA was synthesized using 500 ng of total RNA, MultiScribe reverse transcriptase (Applied Biosystems, Foster City, CA), and oligo(dT) primer (Invitrogen, Carlsbad, CA). The primer sequences (forward and reverse) for *Tex101* (MGI representative transcript sequence NM_019981) and *β-actin* (also known as *Actb*; NM_007393) were as follows: *Tex101* (544 bp PCR product), 5′-TAGACCGTTCCCAGGTCTTG-3′ and 5′-AGCACTGAGTTGTGCCATTG-3′; *β-actin* (UniSTS accession no. 273493; 454 bp PCR product), 5′-ATGGGTCAGAAGGACTCCTA-3′ and 5′-TTGATGTCACGCACGATTTC-3′. PCR was performed using ExTaq DNA polymerase (TaKaRa), 1/20th volume of each RT reaction as template, and a reaction volume of 20 μl in a TaKaRa PCR Thermal Cycler Dice Gradient. The following PCR conditions were used: initial denaturation for 2 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 60 °C, and 1 min at 72 °C, with

a final extension step for 5 min at 72 °C. The PCR products were electrophoresed on 2% agarose gels and stained with SYBR Green I (Molecular Probes).

Immunohistochemistry

TEX101 immunohistochemistry was carried out as described previously (Takayama *et al*., 2005). Briefly, the testes and epididymides from BALB/c mice were fixed for 2 h at room temperature in a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in 100 mM sodium cacodylate buffer (pH 7.4) that contained 5% sucrose. Tissue sections (5 µm thick) were cut with a Microm HM 550 cryostat (Microm, Walldorf, Germany), mounted on 3-aminopropyltriethoxy-silane-coated round glass coverslips, allowed to air-dry, and then washed three times in PBS. Subsequently, the sections were incubated first with 1% bovine serum albumin and 5% normal goat serum in PBS for 1 h at room temperature to block non-specific protein-binding sites and then with the anti-TES101 mAb specific for TEX101 (0.3 µg/ml) for 30 min at 37 °C, followed by at least four washes in PBS. The anti-TES101 mAb was prepared and purified as described previously (Araki & Ikebe 1991; Kurita *et al*., 2001). The sections were then incubated for 30 min at 37 °C with Alexa Fluor-594-labelled goat anti-mouse IgG (10 µg/ml) (Molecular Probes), washed three times in PBS, and counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Molecular Probes), to determine the stage of the seminiferous epithelium cycle during spermatogenesis. After five additional washes in PBS, the sections were mounted in ProLong anti-photobleaching medium on glass microscope slides and examined using a BX60 microscope (Olympus) equipped with a Spot RT SE6 CCD camera (Diagnostic Instruments, Sterling Heights, MI). Control sections received the same treatment, except that the primary antibody was either omitted or replaced with purified non-immune mouse IgG.

Isolated sperm (see *Western blot analysis* below) were fixed for 2 h at room temperature, as described above. In this case, the sperm were in suspension during the first 10 min of fixation; they were then layered over 3-aminopropyltriethoxy-silane-coated round glass coverslips for the remaining incubation time. Subsequently, the coverslips were immunolabelled in the manner described above.

Western blot analysis

Epididymal sperm were prepared by a modification of the method of Métayer *et al*. (2002). Briefly, the epididymides were divided into three zones (caput, corpus and cauda). Samples were cut into slices on pink base plate wax, transferred into microcentrifuge tubes, suspended in 500 µl PBS, and then vortexed gently four times for 5 s. The tubes were then allowed to stand for 1 min, and the supernatants were carefully collected into new tubes. Sperm-rich pellets were obtained from the supernatants by centrifugation (10 s, 8000 *g*). The pellets were resuspended in PBS, washed by two cycles of centrifugation, frozen in liquid nitrogen, and then stored at − 80 °C until use. The relative sample contents of the pellets were confirmed by microscopic examination with differential interference contrast (DIC) imaging. Crude testicular sperm were also prepared in the manner described above.

The pellets were resuspended in octyl-glucopyranoside-based lysate buffer (Takayama *et al*., 2005) and kept on ice for 20 min. After centrifugation (1 min, 8000 *g*), the proteins in the supernatant solution were subjected to SDS-PAGE in 5–20% gradient gels under non-reducing conditions. The proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA) and subsequently probed with the anti-TES101 mAb in 5% low-fat milk in 10 mM TBS that contained 0.1% Tween 20 (Sigma). The signal was detected by the addition of the LumiGLO chemiluminescent substrate reagent (KPL, Gaithersburg, MD) and visualized on Fuji RX-U films (Fuji Photo Film, Tokyo, Japan).

Results

ISH analysis of *Tex101* mRNA expression in adult testis

In the adult testis, hybridization with antisense probes for *Tex101* mRNA gave positive signals in the seminiferous tubules but not in the interstitial cells (Fig. 1).

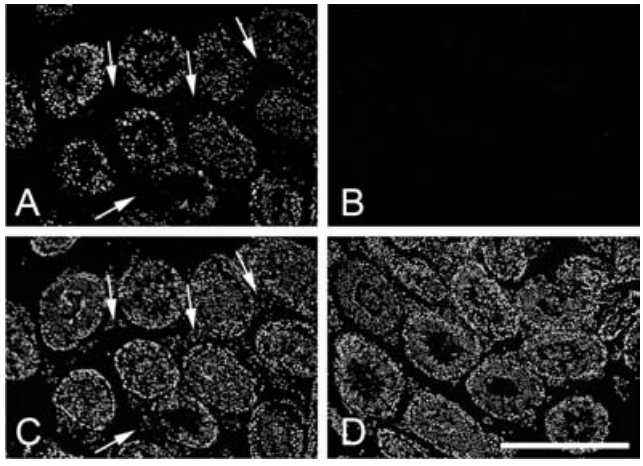

Figure 1 *In situ* analysis of *Tex101* mRNA expression in the adult mouse testis. (*A*) Hybridization with antisense probes for *Tex101* mRNA in the adult testis. The fluorescence signal for expression of *Tex101* mRNA is present in the seminiferous tubules but not in the interstitial cells (arrows). (*B*) Hybridization with sense probes for *Tex101* mRNA. A specific signal is absent in the section probed with the sense probes. (*C*, *D*) Hoechst-33342-stained images of the sections shown in (*A*) and (*B*), respectively. The arrows in (*A*) and (*C*) serve as reference points. Scale bar represents 500 µm. Images *A*–*D* are shown at the same magnification.

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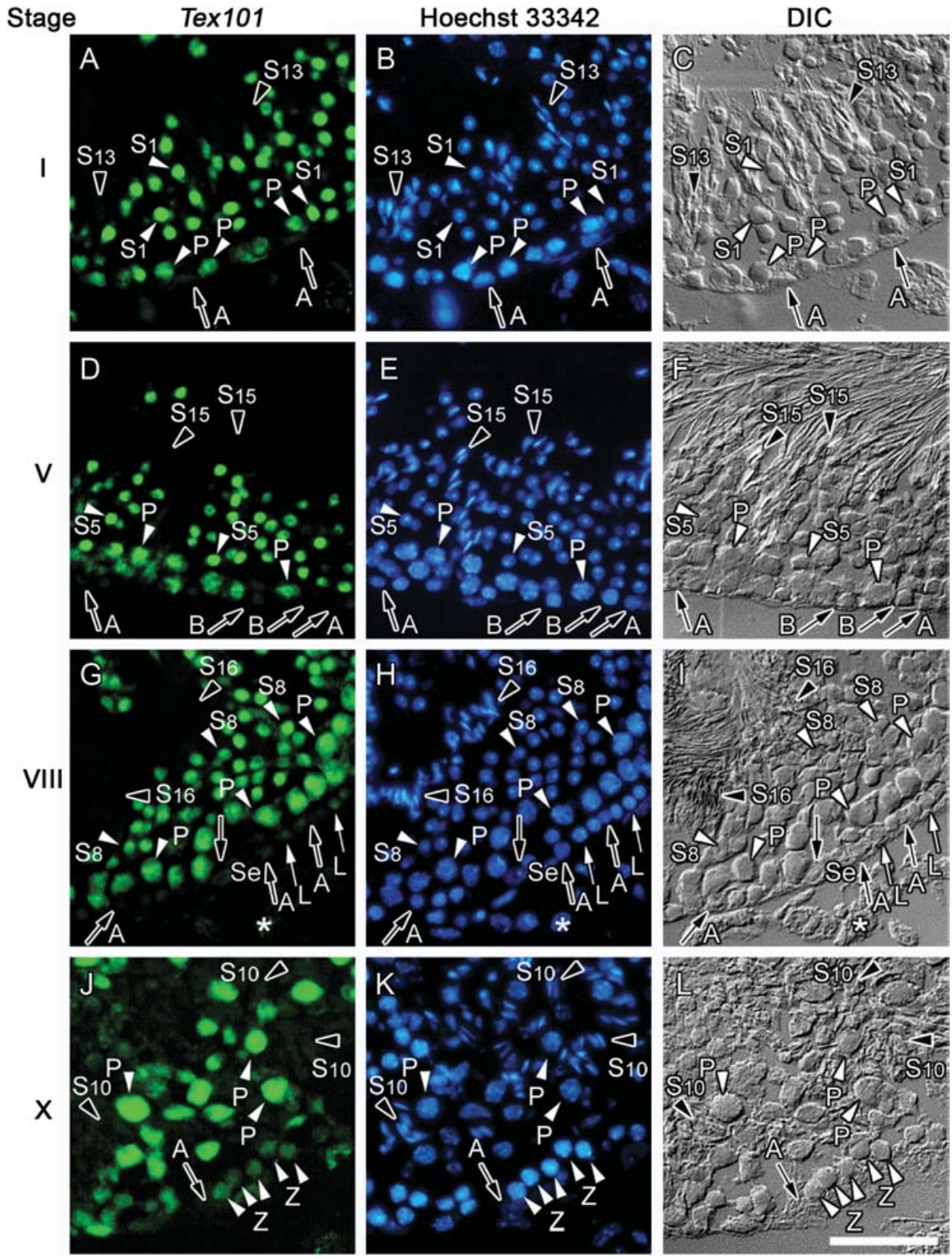

Figure 2 Higher magnification view of *in situ Tex101* mRNA expression in tubules at different stages of spermatogenesis. *Tex101* mRNA expression (green) is detected in cross-sectioned tubules at (*A*) stage I, (*D*) stage V, (*G*) stage VIII and (*J*) stage X. The stages of spermiogenesis are indicated with Arabic numerals, e.g. S_1 and S_{13}. Type A (A) and type B (B) spermatogonia; leptotene (L), zygotene (Z) and pachytene (P) spermatocytes; Sertoli (Se) cells; and interstitial cells (*), including Leydig cells, are evident. Cells positive for *Tex101* mRNA are indicated with white arrows or arrowheads; mRNA-negative cells are indicated with black arrows or arrowheads. (*B*), (*E*), (*H*) and (*K*) are Hoechst-33342 (blue)-stained images of the sections shown in (*A*),

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Specific staining was absent both in the sections that were hybridized with the sense probes (Fig. 1) and in the sections that were pretreated with RNase and then hybridized with the antisense probes (data not shown).

In cross-sectioned mouse seminiferous tubules, 12 stages of spermatid development (numbered I–XII) have been identified, and 16 different steps of spermiogenesis have been recognized (reviewed in Russell *et al*., 1990; de Rooij, 1998). *Tex101* mRNA was present in all the cross-sectioned tubules, appearing first in leptotene-type spermatocytes (Fig. 2*G*). The *Tex101* mRNA was expressed constitutively in differentiating germ cells up to step 9 spermatids, but was no longer detectable in spermatids of steps 10–16 (Fig. 2). *Tex101* mRNA was not found in type A, intermediate, or type B spermatogonia, as shown by ISH (Fig. 2). In addition, neither Sertoli cells nor interstitial cells, which include Leydig cells, expressed histochemically detectable levels of this mRNA (Fig. 2).

RT-PCR analysis of *Tex101* mRNA expression in the adult testis and ovary

To confirm that *Tex101* mRNA was specifically present in the mouse testis, the expression of *Tex101* transcripts in adult testes and ovaries was evaluated by RT-PCR. *Tex101* mRNA was detected in the testis but not in the ovary (Fig. 3). It should be noted that these RT-PCR results are consistent with earlier findings obtained using Northern blotting (Kurita *et al*., 2001).

Immunohistochemical analysis of TEX101 expression in the adult testis and epididymis

Immunohistochemistry was used to verify the expression pattern of TEX101 in the mouse testis. In the testis, TEX101 was observed primarily on cells of the seminiferous tubules (Fig. 4*A*), whereas it was not detected on cells of interstitial tissues, including Leydig cells (data not shown). TEX101 first appeared on the leptotene-type spermatocytes of adult animals and was expressed constitutively on germ cells during spermatogenesis, from spermatocytes to sperm (Fig. 4*A*). In contrast, neither spermatogonia nor Sertoli cells expressed TEX101, as shown by immunohistochemistry (Fig. 4*A*). Isolated testicular sperm showed intense TEX101 immunolabelling along the flagellum, except on the head section (Fig. 4*E*).

The fate of TEX101 expressed on mouse sperm during epididymal transit was investigated by immunohistochemistry. In the proximal portion of the epididymal duct in the caput epididymidis, the sperm stained positively for TEX101 (Fig. 4*B*), whereas in the distal portion of the duct in the caput epididymidis, most of the sperm showed faintly positive to negative staining (Fig. 4*C*). Further investigation showed that TEX101 was also not expressed by sperm in the transport pathway from the duct in the corpus (Fig. 4*D*) and cauda epididymidis to the vas deferens (data not shown). Most of the sperm that were isolated from the cauda epididymidis lacked TEX101 immunostaining (Fig. 4*F*, *F′*). A few sperm from the cauda epididymidis showed weak, dot-like staining near the end of the middle piece of the flagellum (Fig. 4*F*, *F′*). Control sections did not show any specific staining of germ cells (data not shown).

The findings from the ISH and immunohistochemical analyses are summarized in Table 1.

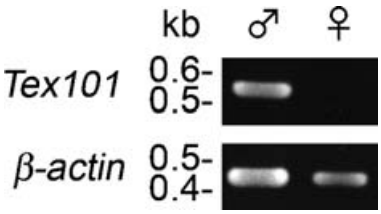

Figure 3 RT-PCR analysis of *Tex101* mRNA expression in the adult mouse testis and ovary. The regions that correspond to *Tex101* or *ß-actin* cDNA fragments are shown.

Western blot analysis of TEX101 expression by epididymal sperm

A testicular sperm extract that was probed with the anti-TES101 mAb showed an intense band of approximately 38 kDa, which is characteristic of the TEX101 protein under non-reducing conditions (Fig. 5). When equal amounts (14.7 μg) of total sperm protein extracts from the three regions of the epididymis (i.e. caput, corpus and cauda) were subjected to Western blotting with the anti-TES101 mAb, a faint TEX101 band was detected in the extracts (Fig. 5).

Discussion

The isolation and identification of cell surface molecules specific for mouse germ cells would provide insights into the detailed mechanisms of germ-cell

(*D*), (*G*) and (*J*), respectively. (*C*), (*F*), (*I*) and (*L*) are differential interference contrast (DIC) images of the sections shown in (*A*), (*D*), (*G*) and (*J*), respectively. The same symbols are presented to provide reference points. Scale bar represents 50 μm. Images *A*–*L* are shown at the same magnification.

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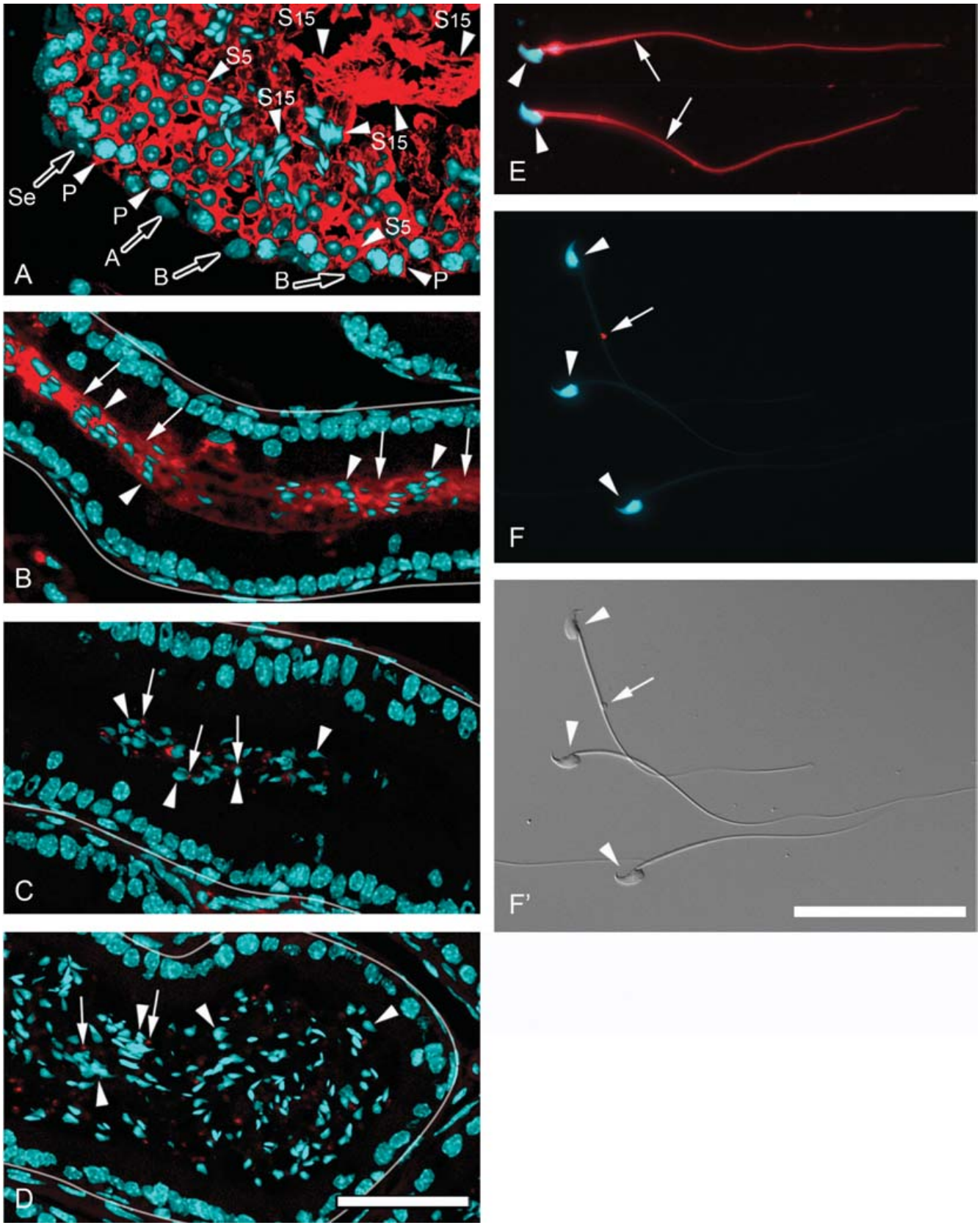

Figure 4 TEX101 immunohistochemistry of the adult mouse testis and epididymis. (*A*) TEX101 localization in the testis. TEX101 (red) is highly expressed in the testis. Type A (A) and type B (B) spermatogonia, pachytene (P)-type spermatocytes, S_5 (S_5) and S_{15} (S_{15}) spermatids, and Sertoli (Se) cells are evident. TEX101-positive cells are indicated with arrowheads;

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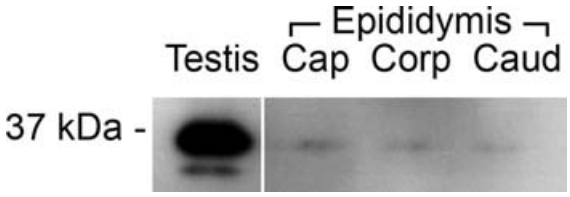

Figure 5 Western blot analysis of TEX101 expression by isolated sperm. Mouse sperm extracts (14.7 μg/lane) from the testis and different epididymal regions were probed with the anti-TEX101 mAb under non-reducing conditions. Cap, caput epididymidis; Corp, corpus epididymidis; Caud, cauda epididymidis.

Table 1 *Tex101* mRNA and TEX101 protein expression profiles in the adult mouse testis and epididymis[a]

	Tex101 mRNA	TEX101
Spermatogonia	–	–
Spermatocytes		
Leptotene	± ~ +	+
Zygotene	+	+
Pachytene	++ ~ + + +	++ ~ + + +
Diplotene	+ + +	++ ~ + + +
Step 1–9 spermatids	+ + +	+ ~ ++
Step 10–16 spermatids	–	++
Sperm in testis	–	++
Sperm in caput epididymidis	ND	++ → – ~ ±
Sperm in corpus epididymidis	ND	– ~ ±
Sperm in cauda epididymidis	ND	– ~ ±
Sperm in vas deferens	ND	– ~ ±
Sertoli cells	–	–
Interstitial cells[b]	–	–
Reference	This study	This study, Kurita *et al*. (2001)

[a] Expression is scored as follows: + + +, strongly positive; ++, moderately positive; +, low-level positivity; ±, barely positive; –, negative. ND, not determined.
[b] Interstitial cells include Leydig cells.

differentiation and sperm maturation, as well as information on fertility disorders caused by the failure of these mechanisms. Using a monoclonal antibody raised against mouse testes, we identified the novel cell surface molecule TEX101, which is expressed specifically on germ cells of the adult mouse testis (Kurita *et al*., 2001). In this study, we clarify the fate of TEX101 expressed on sperm during post-testicular transport using immunohistochemical and Western blot analyses. Recently, we have reported TEX101 expression on germ-cell precursors (prospermatogonia or gonocytes) during male gonadal development (Takayama *et al*., 2005). TEX101 first appeared on prospermatogonia after the primordial germ cells reached the gonadal ridge and subsequently developed into prospermatogonia at around 14 days post-coitus. TEX101 was expressed constitutively on surviving prospermatogonia during prespermatogenesis. After the initiation of spermatogenesis, the prospermatogonia differentiated into spermatogonia. TEX101 expression disappeared from the spermatogonia, but reappeared on spermatocytes, spermatids and testicular sperm. TEX101 was eventually shed from the cell surfaces of epididymal sperm in the transport duct of the caput epididymidis. Moreover, TEX101 was expressed transiently on female germ cells until the start of folliculogenesis in the ovary (Takayama *et al*., 2005). Our data provide evidence that TEX101 is a specific marker for germ cells during gametogenesis. Although the highly regulated, specific and abundant expression of TEX101 on germ cells is considered to be important for gametogenesis, the function of TEX101 remains to be elucidated.

In this study, the expression of *Tex101* mRNA was analysed in detail using ISH. Our low-magnification micrographs of *Tex101* ISH show a differential staining pattern for the cross-sectioned seminiferous tubules (see Fig. 1*A*). This pattern can be interpreted with respect to the various stages of spermatogenesis, in that the expression of *Tex101* mRNA was restricted to certain germ-cell types, i.e. spermatocytes and

TEX101-negative cells are indicated with arrows. DAPI-stained nuclei are evident (blue). (*B–D*) TEX101 expression in: (*B*) the proximal portion of an epididymal duct in the caput epididymidis; (*C*) the distal portion of the same duct in the caput epididymidis; and (*D*) a portion of the same duct in the corpus epididymidis. White lines denote the boundary of the epididymal duct. Epididymal sperm stained with DAPI (arrowheads) are evident in the lumen of the duct. In (*B*), the sperm still exhibit TEX101 staining (arrows), whereas, in (*C*, *D*), most of the sperm show weakly positive (arrows) or negative staining. (*E*, *F*) TEX101 immunolocalization on isolated testicular (*E*) and epididymal (*F*) sperm. (*E*) The isolated testicular sperm show intense TEX101 labelling on the flagellum (arrows). (*F*) One of the sperm isolated from the cauda epididymidis shows faint, dot-like TEX101-staining near the end of the middle piece of the flagellum (arrow). The other sperm lack positive staining. DAPI-stained sperm nuclei are evident (arrowheads). (*F′*) DIC image of the sperm shown in (*F*). The symbols used in both (*F*) and (*F′*) serve as reference points. Scale bars represent 50 μm. Images *A–D* are shown at the same magnification; images *E*, *F* and *F′* are shown at the same magnification.

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step 1–9 spermatids but not spermatogonia. *Tex101* mRNA expression probably starts after the type B spermatogonia enter meiotic prophase. The ISH results presented in this study are in good agreement with previously reported immunocytochemical studies (Kurita *et al*., 2001). The differential synthesis of *Tex101* mRNA and the distribution of TEX101 protein indicate that the persistence of this protein in spermatids from step 10 until step 16 is not the result of *de novo* transcription of *Tex101* (Table 1). Instead, the TEX101 protein remains on the cell surfaces of spermatids and sperm after synthesis. Our RT-PCR analysis shows that *Tex101* mRNA expression is specific to the adult male gonad.

A hydrophilicity/hydrophobicity plot analysis has revealed that mouse TEX101 contains a C-terminal hydrophobic region (Kurita *et al*., 2001). In our preliminary study, testicular sperm TEX101 was released from the cell membrane by phosphatidylinositol-specific phospholipase C treatment (unpublished data). The homologous rat protein, TEC-21, is a glycosylphosphatidylinositol (GPI)-anchored protein (Halova *et al*., 2002). Based on these findings, we hypothesize that the murine sperm TEX101 is a GPI-anchored protein.

The modification of sperm cell surface molecules via sperm-to-epididymis cross-talk contributes significantly to the acquisition of sperm motility and fertilizing ability. Through interactions between the sperm and epididymis, some sperm cell surface molecules, such as angiotensin-converting enzyme (ACE; Métayer *et al*., 2002), are released, and new epididymal secretory molecules, such as CD52 (Kirchhoff, 1996) and complement C4b-binding protein (Nonaka *et al*., 2003), are transported to the cell surface (reviewed in Dacheux *et al*., 2003). Our findings indicate that the sperm cell surface molecule TEX101 is shed from the surfaces of epididymal sperm in the transport duct of the caput epididymidis. However, the molecules that liberate TEX101 from the cell surface remain to be identified. Interestingly, ACE is also expressed on the cell surfaces of testicular sperm and is shed from the mouse sperm membrane during epididymal maturation *in vivo* (Métayer *et al*., 2002). Recently, it has been reported that ACE can cleave GPI-anchored proteins from the cell surfaces of murine epididymal sperm, resulting in the acquisition of egg-binding ability *in vitro* (Kondoh *et al*., 2005). Taking all these findings into consideration, we assume that murine sperm TEX101 is cleaved by activated ACE, which acts as a GPI-anchored protein-releasing factor during epididymal transit, and that TEX101 release may reflect the initial remodelling of the sperm lipid raft domains for the acquisition of fertilization capacity. Further studies are needed to reveal the function(s) of TEX101 and its regulatory roles in gametogenesis and fertilization.

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